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PROCESS FOR PREPARING PRODUCTS USING SOYBEAN PROTEIN AS THE STARTING MATERIAL

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[There are no amendments to this patent.]

Abstract

Objective

To offer a process for preparing products using soybean protein as the starting material for dietary products, cattle feed, aquaculture feed and the like which have a low production

cost, superior production efficiency, can be consumed in large quantities, and, using soybean protein as the starting material, possess superior carcinostatic effects, antiosteoporosis effects, immunosuppressive [sic; immunostimulative] effects, and the like.

Constitution

Characterized by the fact that a product obtained by using soybean protein as the starting material is prepared by preparing a koji through the inoculation of at least one protein selected from extracted soybean protein and separated soybean protein with koji fungi, and producing isoflavone compounds containing a large amount of aglycones* by decomposing the glycosides of the isoflavone compounds in the above-mentioned soybean protein simultaneously with hydrolyzing the protein in the product obtained by the koji preparation treatment by means of adding water to said product.

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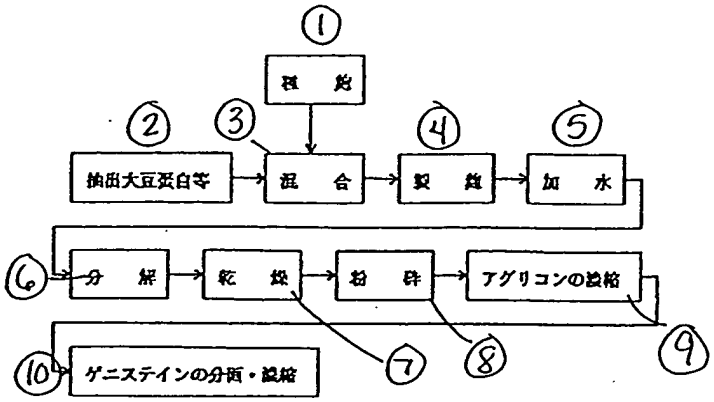
* [Editor's note: The imprecise use of "aglycone" and "glycoside" is inherent in the original document.]



Key: 1 Koji fungus seeding
2 Extracted soybean protein
3 Mixing
4 Koji preparation
5 Hydrolysis
6 Decomposition
7 Drying
8 Pulverizing
9 Aglycone concentration
10 Separation and concentration of genistein fraction

Claims

1. A process for preparing products using soybean protein as the starting material, characterized by the fact that a product obtained by using the above-mentioned soybean protein as the starting material is prepared by preparing a koji through the inoculation of at least one protein selected from extracted soybean protein and separated soybean protein with koji fungi, and producing isoflavone compounds containing a large amount of aglycones by decomposing the glycosides of the isoflavone compounds in the above-mentioned soybean protein simultaneously

with hydrolyzing the protein in the product obtained by the koji preparation treatment by means of adding water to said product.

2. The process for preparing products using soybean protein as the starting material, characterized by the fact that a product with a high concentration of aglycones using the above-mentioned soybean protein as the starting material is prepared by preparing a koji through the inoculation of at least one protein selected from extracted soybean protein and separated soybean protein with koji fungi, producing isoflavone compounds containing a large amount of aglycones by decomposing the glycosides of the isoflavone compounds in the above-mentioned soybean protein simultaneously with hydrolyzing the protein in the product obtained by the koji preparation treatment by means of adding water to said product, and then concentrating the above-mentioned aglycones.

3. The process for preparing products using soybean protein as the starting material, characterized by the fact that a product with a high concentration of genistein using the above-mentioned soybean protein as the starting material is prepared by preparing a koji through the inoculation of at least one protein selected from extracted soybean protein and separated soybean protein with koji fungi, producing isoflavone compounds containing a large amount of aglycones by decomposing the glycosides of the isoflavone compounds in the above-mentioned soybean protein simultaneously with hydrolyzing the protein in the product obtained by the koji preparation treatment by means of adding water to said product, and then separating and concentrating the genistein fraction contained in the above-mentioned aglycones.

4. The process for preparing products using soybean protein

as the starting material, characterized by the fact that a product obtained by using the above-mentioned soybean protein as the starting material is prepared by preparing a koji through the inoculation of at least one protein selected from extracted soybean protein and separated soybean protein with koji fungi, producing isoflavone compounds containing a large amount of aglycones by decomposing the glycosides of the isoflavone compounds in the above-mentioned soybean protein simultaneously with hydrolyzing the protein in the product obtained by the koji preparation treatment by means of adding water to said product, and then removing phytic acid from the above-mentioned soybean protein.

Detailed explanation of the invention

[0001]

Industrial application field

The present invention relates to a process for preparing products using soybean protein as the starting material.

[0002]

In the present invention, the term "soybean protein" stands for extracted soybean protein or separated soybean protein obtained by extraction or separation from soy beans, while the term "products obtained by using soybean protein as the starting material" stands for dietary products, cattle feed, aquaculture

feed, and the like, using the above-mentioned soybean protein as the starting material.

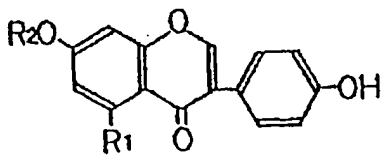
[0003]

Prior art

Generally speaking, isoflavone compounds, such as daidzin, daidzein, genistin, genistein and the like, are contained in soybeans, which are a species of the Leguminosae family.

[0004]

These isoflavone compounds are represented by the following general formula, with their compositions as listed in the following composition table.



Composition table

	R_1	R_2
Daidzin	H	Glucose
Daidzein	H	H
Genistin	OH	Glucose
Genistein	OH	H

Among these isoflavone compounds, daidzein is an aglycone obtained by decomposing the glucose, which is a glycoside, in daidzin, and genistein is an aglycone obtained by decomposing glucose, which is a glycoside, in genistin. [sic]

[0006]

On the other hand, it has been reported that in the process of preparing shoyu and miso, glycosides in soybean isoflavone compounds are hydrolyzed, producing aglycones (Kiyoshi Kihara: Shoken, Vol. 16, No. 5, p. 190 (1990)).

[0007]

However, according to that report, although the hydrolysis of glycosides does advance to a certain degree in the process of steaming of defatted soybeans and in the process of koji preparation, in shoyu lees and soy miso, glycosides practically do not decompose, and it is difficult to use this as a process for preparing dietary products based on using Leguminosae beans as the starting material.

[0008]

Also, there are numerous reports on the pharmacological action of aglycones obtained based on the decomposition of glycosides in isoflavone compounds.

[0009]

For example, it has been clarified that genistein is an inhibitor of tyrosine kinase (TK inhibitor). Because tyrosine kinase is necessary for cancer induction by oncogenes, TK inhibitors have been confirmed to have an oncostatic effect, and their effectiveness has attracted general interest (Akiyama et al.: Seikagaku, Vol. 59, No. 9, p. 1016 (1987)).

[0010]

Also, according to the report made by Hermann Adlerkreutz [transliteration] et al. of Finland (see Am. J. Clin. Nutr. 1991 53 1093-1100 and Clinica Chimica Acta 199:263-278 1991), it has been confirmed that Japanese who consume a large amount of traditional Japanese dietary products, in particular, miso and other soybean products, have a lower risk of breast cancer, prostate cancer, and cancer of the colon, and their mortality rates are low; furthermore, it has been confirmed that the amount of genistein excreted in urine in Japanese who consume a large amount of traditional Japanese dietary products is 32 times greater than that of Europeans and Americans, which indicates that the risk of cancer can be reduced by adjusting one's daily diet.

[0011]

As is evident from the above-mentioned facts, daily intake of dietary products containing aglycones obtained through the decomposition of glycosides of soybean isoflavones, which possess carcinostatic effects, in particular, genistein, is important in terms of preventing cancer.

[0012]

Also, attention has been drawn to the estrogen-like action of isoflavone compounds, which has been confirmed to have antiosteoporosis effects and immunosuppressive effects. In particular, genistein, which is an isoflavonoid aglycone, possesses an estrogen-like action, which makes the suppression of bone mass loss (suppression of bone resorption) possible.

[0013]

This is why numerous suggestions have been made regarding the use of soybean isoflavone compounds in Japanese Kokai Patent Application Nos. Sho 62[1987]-126186, Hei 1[1989]-258669, and Hei 5[1993]-170756.

[0014]

Problems to be solved by the invention

However, most of the isoflavone compounds obtained in accordance with the process described in the above-mentioned

Japanese Kokai Patent Application No. Sho 62[1987]-126186 are daidzin and genistin, which contain glycosides, so that dietary products and the like with the superior pharmacological effects mentioned above cannot be obtained.

[0015]

Also, the process recorded in the above-mentioned Japanese Kokai Patent Application No. Hei 1[1989]-258669 is a process, in accordance with which glycosides in isoflavone compounds are decomposed based on the action of β-glucosidase, which is a kind of enzyme that soybeans contain themselves.

[0016]

Also, the process described in the above-mentioned Japanese Kokai Patent Application No. Hei 5[1993]-170756 is a process for extracting isoflavone compounds from isoflavonoid aglycones produced in shoyu lees or shoyu oil. As described above, although isoflavonoid aglycones are indeed produced in the process of shoyu preparation and the production ratio is quite high, there are the following drawbacks. Namely, because isoflavonoid aglycones are insoluble, they are present in shoyu lees, and because shoyu lees cannot be used as a dietary product, it cannot be used as a process for preparing dietary products. Also, although isoflavonoid aglycones are also produced in soybean miso in the initial stage, the problem is that since soybean miso is a dietary product with a high content of common salt, it cannot be consumed in large quantities.

[0017]

If dietary products containing a large amount of isoflavonoid aglycones, in particular, genistein, which exhibit the above-mentioned superior pharmacological action, could be consumed in large quantities, then it would be possible to enjoy a lifestyle characterized by superior effects in terms of human health preservation and cancer prevention. However, in the past, there were no dietary products that could satisfy this demand.

[0018]

Thus, the discovery of dietary products that could be consumed in large quantities and, using Leguminosae beans as their starting material, possessed superior carcinostatic effects, antiosteoporosis effects, and immunosuppressive effects, has been eagerly awaited. In particular, the discovery of dietary products having the above-mentioned effects has been eagerly awaited in Japan, where the incidence of breast cancer and mortality have been on the rise in recent years because of western influence on dietary habits. Also, the discovery of a preparative method that allows for inexpensively preparing dietary products containing a large amount of isoflavonoid aglycones, in particular, genistein, with high efficiency during the production of such dietary products has been eagerly awaited as well.

[0019]

Also, nowadays, suggestions are being made regarding the use of isoflavonoid aglycones, in particular, genistein, in pharmacology, and the discovery of starting materials containing isoflavonoid aglycones, in particular, genistein, in high concentrations for the manufacture of the such pharmaceutical products has been eagerly awaited as well.

[0020]

Also, the removal of phytic acid, which inhibits the absorption of calcium in the body, from Leguminosae beans is advisable from the standpoint of osteoporosis.

[0021]

Namely, phytic acid is contained in the amount of about 1-2 wt% in soybeans, which are a kind of Leguminosae bean. This phytic acid remains in products obtained using soybeans as the starting material and inhibits the absorption of minerals contained in the products by suppressing the activity of the vitamin B complex contained in the products. More specifically, phytic acid is a compound in which phosphoric acid groups are bound to all the hydroxy groups of myo-inositol, and produces nearly insoluble compounds through chelation with nutritionally important microelement metals. For this reason, people and animals consuming dietary products high in phytic acid have a series of deficiencies because the normal intestinal absorption of these types of metals, for example, calcium, magnesium, iron,

zinc and the like, is inhibited. Also, it has been found that the phytic acid that is present in products containing soybean protein separation products also inhibits the use of zinc in dietary products by monogastric animals. Furthermore, phytic acid is known to possess inhibiting action on various digestive enzymes in the digestive tract, including α-amylase, pepsin, trypsin and the like, which serve as the activating factor for metal ions, such as calcium and the like, which makes its removal from the products highly desirable.

[0022]

However, in the past it was impossible to remove phytic acid in a satisfactory manner.

[0023]

The present invention was made by taking into consideration the above-described circumstances, and its purpose is to offer a process for preparing products using soybean protein as the starting material for low-production-cost dietary products, cattle feed, aquaculture feed and the like, which have superior production efficiency, can be consumed in large quantities, and, using soybean protein as the starting material, have superior carcinostatic effects, antiosteoporosis effects, immunosuppressive effects, and the like.

[0024]

Yet another purpose of the present invention is to offer a process for preparing products using soybean protein as the starting material which are made up of materials containing isoflavonoid aglycones, in particular, genistein, in high concentrations, can be used as the starting material for pharmaceutical products using genistein as the main ingredient, and, moreover, allow for easily and inexpensively producing pharmaceutical products containing genistein in a high concentration.

[0025]

Means to solve the problems

The authors of the present invention conducted in-depth investigations in order to achieve the above-mentioned objectives, and achieved that materials allowing for easily and inexpensively preparing products using soybean protein as the starting material, which include dietary products, cattle feed, aquaculture feed and the like, as well as pharmaceutical products containing isoflavonoid aglycones, in particular, genistein, in high concentrations, can be manufactured inexpensively and with high production efficiency by using as the starting materials, among the four kinds of soybean protein shown in Table I below, extracted soybean protein and separated soybean protein.

[0026]

Namely, among the isoflavone compounds contained in soybeans, the amount contained and content ratios of the combination of daidzin and daidzein and the combination of genistin and genistein contained in soybean protein, including defatted soybeans (soybean lees), extracted soybean protein, separated soybean protein, and concentrated soybean protein, are as shown in Table I below.

[0027]

Table I

	② 脱脂大豆	③ 抽出大豆蛋白	④ 分離大豆蛋白	⑤ 濃縮大豆蛋白
⑥ ダイジン	90	160	74	5.4
⑦ ダイゼイン	5.3	2.5	8.9	検出せず ⑩
⑧ ゲニスチン	120	250	170	8.7
⑨ ゲニステイン	4.4	2.3	16	検出せず ⑩

① (単位：mg/100g)

⑪ (検出限界：0.5mg/100g)

Key: 1 (Units: mg/100 g)
2 Defatted soybeans
3 Extracted soybean protein
4 Separated soybean protein
5 Concentrated soybean protein
6 Daidzin
7 Daidzein
8 Genistin
9 Genistein

10 Not detected
11 Detection limits: 0.5 mg/100 g)

It is evident from Table I that the content of daidzin and genistin is high, and the content of daidzein and genistein, which are their aglycones, is low. Then, among the four kinds of soybean protein shown in Table I, there is about 1.8 times as much daidzin and about 2.1 times as much genistin in the extracted soybean protein as in defatted soybeans, and about 1.4 times as much genistin in separated soybean protein as in defatted soybeans, so that, by using them as the starting material, it is possible to prepare products using soybean protein as the starting material inexpensively and with high efficiency. From the standpoint of the manufacturing process, which will be described later, extracted soybean protein and separated soybean protein contain a large amount [of isoflavanoids] in a state in which water-soluble glycoside isoflavonoid compounds are concentrated, and, by means of converting these glycoside isoflavone compounds to aglycones by using koji fungi, it is possible to produce isoflavone compounds containing a large amount of aglycones in high yield and inexpensively. Also, when using soybeans containing more isoflavone compounds than ordinary soybeans due to using soybeans, from which the extracted soybean protein and separated soybean protein are extracted, chosen from a variety that has been changed by genetic enhancement to contain a higher amount of isoflavone compounds, the above-mentioned extracted soybean protein and separated soybean protein, which serve as the starting materials in the preparative method of the present invention, are made to contain a larger amount of isoflavone

compounds, so that isoflavone compounds containing an even greater amount of aglycones in the products produced in accordance with the present invention can be prepared in high yield and inexpensively.

[0028]

If materials are obtained which contain isoflavonoid aglycones, in particular, genistein in high concentrations based on concentrating the isoflavone compounds containing a large amount of aglycones prepared by doing so, these materials can be used as the starting material for pharmaceutical products using genistein as the main ingredient, and, moreover, pharmaceutical products containing genistein in a high concentration can be prepared easily and inexpensively.

[0029]

Based on this research, to achieve the above-mentioned purpose, the process according to Claim 1 of the present invention for preparing products using soybean protein as the starting material is characterized by the fact that a product obtained by using the above-mentioned soybean protein as the starting material is prepared by preparing a koji through the inoculation of at least one protein selected from extracted soybean protein and separated soybean protein with koji fungi, and producing isoflavone compounds containing a large amount of aglycones by decomposing the glycosides of the isoflavone compounds in the above-mentioned soybean protein simultaneously

with hydrolyzing the protein in the product obtained by the koji preparation treatment by means of adding water to said product.

[0030]

The process according to Claim 2 of the present invention for preparing products using soybean protein as the starting material is characterized by the fact that a product with a high concentration of aglycones using the above-mentioned soybean protein as the starting material is prepared by preparing a koji through the inoculation of at least one protein selected from extracted soybean protein and separated soybean protein with koji fungi, producing isoflavone compounds containing a large amount of aglycones by decomposing the glycosides of the isoflavone compounds in the above-mentioned soybean protein simultaneously with hydrolyzing the protein in the product obtained by the koji preparation treatment by means of adding water to said product, and then concentrating the above-mentioned aglycones.

[0031]

The process according to Claim 3 of the present invention for preparing products using soybean protein as the starting material is characterized by the fact that a product with a high concentration of genistein using the above-mentioned soybean protein as the starting material is prepared by preparing a koji through the inoculation of at least one protein selected from extracted soybean protein and separated soybean protein with koji fungi, producing isoflavone compounds containing a large amount of aglycones by decomposing the glycosides of the isoflavone

compounds in the above-mentioned soybean protein simultaneously with hydrolyzing the protein in the product obtained by the koji preparation treatment by means of adding water to said product, and then separating and concentrating the genistein fraction contained in the above-mentioned aglycones.

[0032]

The process according to Claim 4 of the present invention for preparing products using soybean protein as the starting material is characterized by the fact that a product obtained by using the above-mentioned soybean protein as the starting material is prepared by preparing a koji through the inoculation of at least one protein selected from extracted soybean protein and separated soybean protein with koji fungi, producing isoflavone compounds containing a large amount of aglycones by decomposing the glycosides of the isoflavone compounds in the above-mentioned soybean protein simultaneously with hydrolyzing the protein in the product obtained by the koji preparation treatment by means of adding water to said product, and then removing phytic acid from the above-mentioned soybean protein.

[0033]

Function

According to the process of Claim 1 of the present invention for preparing products using soybean protein as the starting material, isoflavone compounds containing a large amount of aglycones can be produced by decomposing the glycosides of the

isoflavone compounds in soybean protein by allowing koji fungus to grow by means of preparing a koji through the inoculation of soybean protein with koji fungi, and, further, by decomposing the glycosides of the isoflavone compounds in the above-mentioned soybean protein simultaneously with hydrolyzing the protein in said product obtained through the koji preparation treatment by means of adding water to said product.

[0034]

According to the process of Claim 2 of the present invention for preparing products using soybean protein as the starting material, a material with a high concentration of aglycones can be produced by concentrating aglycones in the isoflavone compounds containing a large amount of aglycones prepared in accordance with Claim 1.

[0035]

According to the process of Claim 3 of the present invention for preparing products using soybean protein as the starting material, a material with a high concentration of genistein can be produced by separating and concentrating the genistein fraction of the isoflavone compounds containing a large amount of aglycones prepared in accordance with Claim 1.

[0036]

According to the process of Claim 4 of the present invention for preparing products using soybean protein as the starting

material, phytic acid can be removed from the soybean protein simultaneously with decomposing the glycosides of the isoflavone compounds in the Leguminosae beans by allowing koji fungi to grow by means of preparing a koji through the inoculation of soybean protein with koji fungi, and, further, phytic acid can be removed from the soybean protein along with producing isoflavone compounds containing a large amount of aglycones by decomposing the glycosides of the isoflavone compounds in the above-mentioned soybean protein simultaneously with hydrolyzing the protein in said products.

[0037]

If the process of the present invention is used in the above manner, isoflavone compounds containing a large amount of aglycones can be obtained in high yield and inexpensively because of using extracted soybean protein and separated soybean protein with a high content of isoflavone compounds. Based on this, it is possible to inexpensively prepare dietary products with superior carcinostatic effects containing a large amount of isoflavonoid aglycones, in particular, genistein. Also, if a material is obtained which contains isoflavonoid aglycones, in particular, genistein, in high concentrations, this material can be used as a starting material for pharmaceutical products using genistein as the main ingredient, and, moreover, pharmaceutical products containing genistein in high concentrations can be prepared easily and inexpensively. Also, phytic acid can be simultaneously removed from the soybean protein.

[0038]

Application examples

Below, explanations are provided with respect to application examples of the present invention with reference to Figure 1.

[0039]

Figure 1 is a flow chart that shows a first application example of a preparative method for products obtained by producing isoflavone compounds containing a large amount of aglycones based on the process of this invention, by decomposing the glycosides of the isoflavone compounds contained in soybean protein, which include at least one kind of protein from among extracted soybean protein and separated soybean protein, as well as an application example of a preparative method for preparing materials containing isoflavonoid aglycones, in particular, genistein, in high concentrations, and, along with that, a first application example of a preparative method for products in which phytic acid has been removed from the soybean protein, in which the products obtained by producing isoflavone compounds containing a large amount of aglycones are subjected to aglycone concentration or separation and concentration of the genistein fraction to prepare materials containing isoflavonoid aglycones, in particular, genistein, in high concentrations. Namely, the inventions recorded in the above Claims 1-4 can be used based on the same preparative method.

[0040]

First of all, explanations are provided with respect to the invention of Claim 1, namely, with respect to the case of preparing products obtained by producing isoflavone compounds containing a large amount of aglycones.

[0041]

In accordance with the procedure shown in Figure 1, first of all, at least an extracted soybean protein or separated soybean protein serving as the starting material is prepared.

[0042]

One of them, the extracted soybean protein is obtained by preparing an aqueous extract of defatted soybeans, centrifuging, concentrating under reduced pressure and spray drying. By this extraction the water-soluble glycoside isoflavone compounds contained therein are extracted from the defatted soybeans, and introduced in large quantities in the extracted soybean protein in a highly concentrated condition. Commercially available protein, for example, such as SORUPII-NY [transliteration] from Nisseiseiyu K.K., and the like, can be used as said extracted soybean protein. Because this extracted soybean protein is in powder form, in order to conduct the koji preparation treatment of the present invention, which will be described later, in an efficient manner, it is advisable to make granules with a diameter of from 1-2 mm to 10 mm by performing agitation while sparingly adding water. For example, 200 mL water can be added

per 200 g extracted soybean protein. Also, an excellent mycelium of koji fungi can be produced by swelling molding [unconfirmed translation] of soybean protein by means of an extruder, or the like. Furthermore, bulk pieces can be formed by adding water to soybean protein powder, kneading, and then molding it in the form of plates or rods.

[0043]

The other material, that is, separated soybean protein, is obtained by subjecting defatted soybeans to extraction using water or dilute alkali (0.02-01% sodium hydroxide), removing insoluble matter by centrifugation, and then adjusting the pH to 4.2-4.5 and subjecting the protein to isoelectric precipitation. After washing the separated matter obtained by centrifuging this precipitated material, it is neutralized using sodium hydroxide, dissolved, heated and spray dried. In the process of said water or dilute alkali-based extraction, water-soluble glycoside isoflavone compounds contained in defatted soybeans are extracted therefrom, and introduced in large quantities in the extracted soybean protein in a concentrated form. Commercially available protein, such as the protein from Fuji Purina Protein K.K. known under the trade name FujiPro E, and the like, can be used as such separated soybean protein. Because said separated soybean protein is also in powder form (in the same manner as the above-described extracted protein) in order to effectively conduct the koji preparation treatment of the present invention, which will be described later, it is advisable to make granules with a diameter of from 1-2 mm to 10 mm by performing agitation while sparingly adding water. The corresponding amounts of separated soybean

protein and water may be the same as in the above-described case of extracted soybean protein.

[0044]

At least one protein selected from extracted soybean protein and separated soybean protein prepared in the above-described manner as the starting materials are used in the method of the present invention in the manner described below.

[0045]

Namely, a specified amount of a mixture obtained by mixing koji seeding culture made up of koji fungi with a powder of extracted soybean protein in a specified weight ratio, is added to extracted soybean protein, and made into granules by adding water in the above-described manner, and the two are mixed together until the mixture becomes homogeneous while adjusting the water content so that the entire water content is 35-50 wt%, preferably, 42-44 wt%. As for their weight ratio, for example, 0.3 g koji fungi per 50 g powder of extracted soybean protein can be mixed in a material obtained by adding 200 mL water per 200 g extracted soybean protein and stirred. Koji preparation can be conducted so that contamination with extraneous fungi other than koji fungi is prevented by lowering the surface water activity [sic] by means of adding dry powders of extracted soybean protein to powders of extracted soybean protein containing a large amount of water.

[0046]

After that, the mixture is placed inside an apparatus for preparing koji and kept there for a specified time, heated to a temperature of about 28-32°C, and, upon inoculating the extracted soybean protein having a low water content, such as 35-50 wt%, and, preferably, 42-44 wt%, with koji fungi, the preparation of a koji is performed until a sufficient amount of enzymes necessary for the production of aglycones by decomposing the glycosides of the isoflavone compounds in the extracted soybean protein is produced.

[0047]

In this case, the enzyme that decomposes glycosides in isoflavone compounds and is called β-glucosidase, which is made by the koji fungi in the process of growth of the koji fungi in the extracted soybean protein, and produces isoflavonoid aglycones by decomposing the glycosides of the isoflavone compounds in the extracted soybean protein.

[0048]

Koji fungi that have been traditionally used specifically in Japanese fermented dietary products and tempeh, including nutritionally safe *Aspergillus usami*, *Aspergillus kawachi*, *Aspergillus awamori*, *Aspergillus* Saitoi [transliteration], *Aspergillus oryzae*, and *Aspergillus niger*. Other *Aspergillus* and *Rhizopus* fungi can be used as the koji fungi for the preparation of the koji.

[0049]

As for the duration of fermentation, depending on the type of the koji fungi used, the duration of fermentation is at least not less than 24 h and should be sufficient to completely decompose the glycosides in the isoflavone compounds of the extracted soybean protein.

[0050]

Next, after adding water to the product obtained after the preparation of the koji so that the water content is about 50 wt%, the mixture is kept heated to a temperature of 30-65°C, and preferably, about 50°C for a specified time, and hydrolysis is conducted while producing isoflavonoid aglycones by means of sufficiently reducing the amount of glycosides in the isoflavone compounds contained in the extracted soybean protein based on the decomposing action of the β-glucosidase contained in the produced material.

[0051]

As for the protein hydrolysis, the duration of the hydrolysis and the temperature of the hydrolysis must be sufficient to sufficiently reduce the amount of glycosides in the isoflavone compounds in the extracted soybean protein, in accordance with the type of the koji fungi used.

[0052]

Table II shows, for Application Example 1, the content of isoflavone compounds in extracted soybean protein obtained by conducting koji preparation for 48 h at a temperature of 30°C using a starting material produced by mixing 0.3 g koji fungi (*Aspergillus niger*) per 50 g powder of extracted soybean protein in a material obtained by adding 200 mL water per 200 g extracted soybean protein and stirring, then adding the same amount of water by weight as the weight of the product and performing protein hydrolysis for 48 h at a temperature of 50°C; and for Application Example 2, [Table II shows] the content of isoflavone compounds in separated soybean protein obtained by conducting koji preparation for 48 h at a temperature of 30°C using a starting material produced by mixing 0.3 g koji fungi (*Aspergillus niger*) per 50 g powder of separated soybean protein in a material obtained by adding 200 mL water per 200 g separated soybean protein and stirring, then adding the same amount of water by weight as the weight of the product and performing protein hydrolysis for 48 h at a temperature of 50°C, and in a comparative example, the content of isoflavone compounds in defatted soybeans obtained by conducting koji preparation for 48 h at a temperature of 30°C using koji fungi (*Aspergillus niger*) on defatted soybeans in the same manner as in the present invention, then adding the same amount of water by weight as the weight of the product and performing protein hydrolysis for 48 h at a temperature of 30°C.

[0053]

By doing so, in the initial stage, organic acids are produced and the growth of extraneous fungi in the extracted soybean protein is suppressed, there is no danger of secondary contamination, and products obtained using extracted soybean protein as the starting material can be produced in large quantities. Also, even without a low water content, treatment sufficiently reducing the amount of glycosides in isoflavone compounds can be conducted.

[0054]

Table II

(2) (3) (1)(単位：mg/100g)

	比較例	実施例1	実施例2 (3)
(4)	脱脂大豆	抽出大豆蛋白	分離大豆蛋白 (6)
(7) ダイジン	(8)検出せず	(8)検出せず	(8)検出せず (5)
ダイゼイン	70	124	67
ゲニスチン	1.3	5.5	5.4
ゲニステイン	64	203	133.1

(9)(検出限界：0.5mg/100g)

Key: 1 (Units: mg/100 g)
2 Comparative example
3 Application Example
4 Defatted soybeans
5 Extracted soybean protein
6 Separated soybean protein

7 Daidzin
Daidzein
Genistin
Genistein
8 Was not detected
9 (Detection limits: 0.5 mg/100 g)

According to Table II, in the comparative example, in which untreated defatted soybeans were used as the starting material, the content of daidzein and genistein, which are aglycones of isoflavone compounds, was 70 mg and 64, exhibiting a significant increase of about 13.3 times and 14.5 times in comparison with the content in untreated defatted soybeans shown in Table I. By comparison with the comparative example, in Application Example 1 of the present invention, in which extracted soybean protein was used as the starting material, the content of daidzein and genistein, which are aglycones of isoflavone compounds, was 124 mg and 203 mg [respectively], also exhibiting a significant increase of about 1.8 times and 3.2 times as compared with the comparative example. Also, in Application Example 2, in which separated soybean protein was used as the starting material, the content of daidzein, which is one of the aglycones of isoflavone compounds, was 67 mg, which was practically equal to the content in the above-mentioned comparative example, while the content of the other aglycone, genistein, was 133.1 mg, that is, about 2.1 times greater than the content of the comparative example, which constitutes another significant increase.

[0055]

In this manner, based on the present invention, aglycones, which possess powerful pharmacologic action among the isoflavone compounds of soybeans, can be prepared with an extremely high production ratio.

[0056]

In particular, in Application Example 1 and Application Example 2, genistein, which possesses powerful carcinostatic effects, can be prepared with an extremely high production ratio, and when health foods with added genistein are prepared, it is possible to inexpensively offer products with powerful carcinostatic effects containing a large amount of genistein, whose manufacture is simple, and whose starting materials used in each application example are easily obtainable.

[0057]

Next, explanations are provided with reference to figures with respect to the inventions recorded in Claim 2 and Claim 3, in other words, with respect to the cases of preparing materials containing isoflavonoid aglycones, in particular, genistein, in high concentrations.

[0058]

As shown in Figure 1, the production processes of the inventions recorded in Claim 2 and Claim 3 allow for producing

isoflavone compounds containing large amounts of aglycones based on the fact that the koji preparation process, hydrolysis process, and decomposition process, which are the processes of the invention recorded in the above-described Claim 1, are performed in the same manner.

[0059]

After that, in the invention of Claim 2, a material containing isoflavonoid aglycones in high concentrations is prepared by conducting the concentration of aglycones using the isoflavone compounds with a large amount of aglycones produced by doing so. In this case, as shown in Figure 1, after the decomposition process, by drying and pulverizing the product, the subsequent concentration of aglycones is performed in an efficient manner. Naturally, the process of drying and the process of pulverizing the product can be omitted.

[0060]

Also, in the invention recorded in Claim 3, the concentration of aglycones is conducted, and then the separation and concentration of the genistein fraction thereof is performed, preparing a material containing genistein in a high concentration.

[0061]

The concentration of aglycones as well as the separation and concentration of the genistein fraction can be conducted in

combination with one or several well-known means, such as extraction using organic solvents, nonionic adsorption resins, freeze-drying, concentration under reduced pressure, and the like.

[0062]

The above-mentioned extraction based on using organic solvents consists in using organic solvents to extract aglycones from the isoflavone compounds containing a large amount of aglycones produced from extracted soybean protein in accordance with Claim 1. Alcohols, water-containing alcohols, ethyl ether, ethanol, ethyl acetate, chloroform, methyl isobutyl ketone, butanol and the like are suggested as said organic solvents. Based on this method, aglycones made up of daidzein and genistein are concentrated together without separating into fractions.

[0063]

The above-mentioned nonionic adsorption resins are resins used in column chromatography, in which isoflavone compounds containing a large amount of aglycones produced from extracted soybean protein and the like in accordance with Claim 1 are adsorbed on the column resin, and, after that, aglycones are eluted by separating them into the daidzein and genistein fraction based on the difference in the rate of elution. The above-mentioned column resins can be any resins, as long as they allow for elution with separation into the daidzein and genistein fractions, for example, one can suggest using porous

styrene-divinylbenzene resins (Mitsubishi Chemical Industries, Ltd. trade name: Daiyaion [transliteration] PH-20, Rohm & Haas Co. trade name: Amberlite XAD-2, XAD-4, Sumitomo Chemical Co., Ltd. trade name: Duolite S-861, S862).

[0064]

By means of continuously conducting the concentration of aglycones by performing extraction based on the above-mentioned organic solvents and elution with separation into the daidzein fraction and genistein fraction based on the above-mentioned high-speed liquid chromatography, it is possible to produce materials targeting genistein, in which genistein is concentrated in very high concentrations.

[0065]

By concentrating isoflavone compounds containing a large amount of aglycones by doing so, it is possible to obtain materials containing isoflavonoid aglycones, in particular, genistein, in high concentrations; these materials can be used as the starting material for pharmaceutical products whose main ingredient is genistein, and, moreover, pharmaceutical products containing genistein in high concentrations can be produced easily and inexpensively.

[0066]

Next, explanations are provided with respect to the invention recorded in Claim 4, in other words, the case of

preparing products obtained by removing phytic acid from extracted soybean protein along with preparing products with isoflavone compounds containing a large amount of aglycones.

[0067]

The production process of the invention recorded in Claim 4 is conducted in the same manner as the production process of the invention recorded in Claim 1 described above; however, in the koji preparation process, hydrolysis process and decomposition process, phytic acid is removed from extracted soybean protein along with producing isoflavone compounds containing a large amount of aglycones.

[0068]

Below, explanations are provided with respect to each of these processes.

[0069]

In the koji preparation process, a mixture of extracted soybean protein or such and the koji fungi is placed in an apparatus for preparing koji, kept there for a specified amount of time heated at a temperature of 28-32°C, and, upon inoculating the extracted soybean protein having a low water content, such as 35-50 wt%, and, preferably, 42-44 wt%, with koji fungi, the preparation of a koji is performed until a sufficient amount of enzymes necessary for sufficiently reducing the amount of phytic acid in the extracted soybean protein is produced.

[0070]

In this case, enzymes decomposing phytic acid, called phytase and phosphatase, made by the koji fungi, are produced in the extracted soybean protein due to the growth of the koji fungi in the extracted soybean protein.

[0071]

Namely, the above-mentioned phytic acid is removed based on the production of one or several acids, such as inositol-5-phosphoric acid, inositol-4-phosphoric acid, inositol-3-phosphoric acid, inositol-2-phosphoric acid, inositol-1-phosphoric acid and inositol through the release of phosphoric acid groups by the enzymes decomposing phytic acid from phytic acid, which is a compound, in which phosphoric acid groups are bound to all the hydroxy groups of myoinositol.

[0072]

Koji fungi that have been traditionally used specifically in Japanese fermented dietary products and tempeh, including nutritionally safe *Aspergillus usami*, *Aspergillus kawachi*, *Aspergillus awamori*, *Aspergillus saitoi*, *Aspergillus oryzae*, *Aspergillus niger*, and other *Aspergillus* and *Rhizopus* fungi with a high phytase titer and phosphatase titer can be used as the koji fungi used for the preparation of the koji.

[0073]

As for the duration of fermentation, depending on the type of the koji fungi used, the duration of fermentation is at least not less than 24 h, and should be sufficient to completely decompose glycosides in the isoflavone compounds of the extracted soybean protein.

[0074]

In the subsequent hydrolysis process and decomposition process, after adding water to the product obtained after the termination of koji preparation, it is kept at a temperature of 30-65°C, and preferably, about 50°C for a specified time, hydrolysis is conducted, and the amount of phytic acid contained in the extracted soybean protein is sufficiently reduced based on the decomposing action of the phytase and phosphatase contained in the produced material.

[0075]

As for the protein hydrolysis, the duration of the hydrolysis and the temperature of the hydrolysis have to be sufficient to sufficiently reduce the amount of phytic acid in the extracted soybean protein, in accordance with the type of the koji fungi used.

[0076]

Also, as far as the removal of phytic acid is concerned, it is conducted by eliminating at least one phosphoric acid group from phytic acid made up of inositol-6-phosphoric acid, but inositol-4-phosphoric acid, inositol-3-phosphoric acid, inositol-2-phosphoric acid, and inositol-1-phosphoric acid, obtained by releasing at least 2 phosphoric acid groups, possess solubility in water and action that significantly stimulates the absorption of minerals, such as calcium and the like, which are contained in the products obtained by using soybean protein as the starting material.

[0077]

More specifically, the above-mentioned inositol-6-phosphoric acid and inositol-5-phosphoric acid are characterized by strong ionic bonds, so that the elution of calcium cannot take place, which greatly suppresses the calcium-absorbing action. By contrast, acids from inositol-4-phosphoric acid to inositol-1-phosphoric acid, along with bonding calcium quite well, possess appropriate affinity allowing for easily releasing bonded calcium when necessary, and exhibit the characteristic action of promoting the absorption of calcium, which was mentioned above.

[0078]

Therefore, products with a more efficient absorption of minerals are obtained by removing phytic acid by obtaining one or several acids, such as inositol-5-phosphoric acid,

inositol-4-phosphoric acid, inositol-3-phosphoric acid, inositol-2-phosphoric acid, inositol-1-phosphoric acid and inositol, by means of releasing at least 2 phosphoric acid groups from phytic acid made up of inositol-6-phosphoric acid. In this case, the number of phosphoric acid groups released from phytic acid may be controlled by adjusting fermentation time and hydrolysis time, as well as hydrolysis temperature in accordance with the type, condition, characteristics and relative amount of the soybean protein, the type, condition, characteristics and relative amount of the koji fungi, and the type and characteristics of the product.

[0079]

Table III shows the content of phytic acid in the case of untreated soybean lees, in the case of extracted soybean protein A and extracted soybean protein B obtained by first using two kinds of koji (A is *Aspergillus niger*, and B is *Aspergillus awamori*) to conduct koji preparation at a temperature of 30°C for 48 h using extracted soybean protein, and then conducting protein hydrolysis at a temperature of 50°C for 48 h by adding water in such an amount that it has the same weight as the weight of each product, and in the case of soybeans lees obtained as a result of the conventional procedure of washing with alcohol.

[0080]

Table III

① 対象大豆粕	フィチン酸含有量(mg/100g) ②
③ 無処理大豆粕	999 (mg/100g)
④ 焼酎麹処理A	検出せず ⑤
⑥ 焼酎麹処理B	検出せず
⑦ アルコール洗浄大豆粕	1150 (mg/100g)

⑧ (検出限界：5mg/100g)

Key: 1 Soybean lees subjected to treatment
2 Content of phytic acid (mg/100 g)
3 Untreated soybean lees
4 Treated with koji A
5 Not detected
6 Treated with koji B
7 Soybean lees washed with alcohol
8 (Detection limits: 5 mg/10g)

According to Table III, while the content of phytic acid in untreated soybean lees was 999 mg, that is, about 1%, the content of phytic acid in extracted soybean protein A and extracted soybean protein B (obtained by conducting koji treatment in accordance with the method of the present invention, and then conducting protein hydrolysis at a temperature of 50°C for 48 h by adding water in such an amount that it has the same weight as the weight of each product) was so small that it was not even detectable, in other words, the amount of phytic acid was reduced so much that it was practically completely decomposed.

[0081]

On the other hand, the content of phytic acid in soybean lees obtained by conducting the conventional procedure of washing with alcohol was 1150 mg, which means that it had not decreased at all.

[0082]

In this manner, based on the present invention, it is possible to prepare genistein, which possesses a powerful pharmacological action among isoflavone compounds contained in extracted soybean protein, with an extremely high production ratio, and, at the same time, to reduce the amount of phytic acid in the extracted soybean protein to a great extent or practically completely.

[0083]

Also, materials containing isoflavonoid aglycones, in particular, genistein in a high concentration can be obtained easily and inexpensively.

[0084]

As was explained above, because the products using extracted soybean protein as the starting material prepared in accordance with the present invention are prepared without adding common salt, the dietary products obtained are extremely low in salt content, and when used, they can be consumed in large quantities.

Then, large quantities of isoflavonoid aglycones, which possess carcinostatic effects, antiosteoporosis effects, immunosuppressive effects, are contained in such products, which allows for creating a diet exhibiting superior effects in terms of maintaining human health and preventing the development of cancer.

[0085]

Furthermore, because extracted soybean protein and separated soybean protein, which are high in isoflavone compounds, are used as the starting materials in accordance with the present invention, isoflavone compounds containing a large amount of aglycones can be produced in high yields and inexpensively, and, based on this, it is possible to inexpensively prepare dietary products with superior carcinostatic effects containing a large amount of isoflavonoid aglycones, in particular, genistein.

[0086]

Furthermore, based on the present invention, materials containing isoflavonoid aglycones, in particular, genistein, in high concentrations can be obtained easily and inexpensively, and for this reason, such materials can be used as the starting material for pharmaceutical products using genistein as their main ingredient, and, moreover, pharmaceutical products containing genistein in high concentrations can be prepared easily and inexpensively.

[0087]

In particular, people who do not have enzymes that decompose isoflavones in the colon can absorb genistein directly by consuming the dietary products and pharmaceutical products of the present invention containing genistein in high concentrations, and, based on this, they can enjoy a lifestyle characterized by superior effects in terms of maintaining health and in terms of cancer prevention.

[0088]

Also, from the standpoint of osteoporosis, on the one hand, due to the effect of preventing bone mass loss exhibited by isoflavonoid aglycones, and on the other hand, due to the removal of phytic acid, the activity of the useful vitamin B complex and the like possessing a development-promoting action and lipotropic action is maintained at a high level, and the effect of promoting the absorption of calcium contained in said extracted soybean protein or such is displayed, and, furthermore, these effects are displayed in a synergic fashion, based on which these dietary products possess extremely superior antiosteoporosis effects. In particular, effects are displayed when they are used for dietetic therapy in people easily developing osteoporosis on a hormonal basis.

[0089]

Also, when extracted soybean protein prepared by doing so is used as feed and the like, as shown in Figure 1, by drying and

then pulverizing the extracted soybean protein prepared in accordance with the above-described application examples, a powder of extracted soybean protein possessing powerful pharmacological action is obtained and can be used as the starting material for cattle feed and aquaculture feed. Also, to produce such products used as the starting materials for cattle feed and aquaculture feed in a less expensive manner, it is possible to use defatted soybeans as the starting material, as in the comparative example shown in the above-mentioned Table II.

[0090]

In this manner, because in accordance with the inventions recorded in Claim 1 and Claim 2, by growing a live culture of koji fungi, aglycones possessing a powerful pharmacological action among isoflavone compounds contained in extracted soybean protein are produced with an extremely high production ratio, phytic acid is removed from the extracted soybean protein, and, furthermore, the protein is hydrolyzed, aglycones can be produced and phytic acid removed easily even if the extracted soybean protein is in a solid or liquid form, their production process is simple, and production costs are low. Then, because materials containing isoflavonoid aglycones, in particular, genistein, in high concentrations can be easily and inexpensively prepared in the inventions recorded in Claim 3 and Claim 4 by using the product inexpensively prepared in accordance with the invention recorded in Claim 1, these materials can be used as the starting materials for pharmaceutical products using genistein as their main ingredient, pharmaceutical products containing genistein in high concentrations can be prepared easily and inexpensively.

[0091]

Also, the conventional apparatus for preparing koji can be used as is in the present invention, there is no need to manufacture equipment for production, so that the invention possesses a high degree of universality.

[0092]

In addition, the present invention is not limited to the above-described application examples, and can be modified as the occasion demands.

[0093]

Effect of the invention

Because the present invention has the above constitution and operation, it allows for preparing dietary products, cattle feed and aquaculture feed with superior carcinostatic effects, antiosteoporosis effects, immunosuppressive effects and the like by using soybean protein as the starting material. Also, because the protein is hydrolyzed, the dietary products, cattle feed and aquaculture feed are easy to digest, and are easily absorbed, based on which they are dietetically superior, offering a high efficiency of protein utilization, and, moreover, can be consumed in large quantities because no common salt is added thereto. Furthermore, because extracted soybean protein with a high content of isoflavone compounds is used as the starting material, the dietary products, cattle feed and aquaculture feed can be

prepared inexpensively, with superior production efficiency. In particular, the invention has the effect allowing to inexpensively prepare dietary products with superior carcinostatic effects containing a large amount of isoflavonoid aglycones, in particular, genistein.

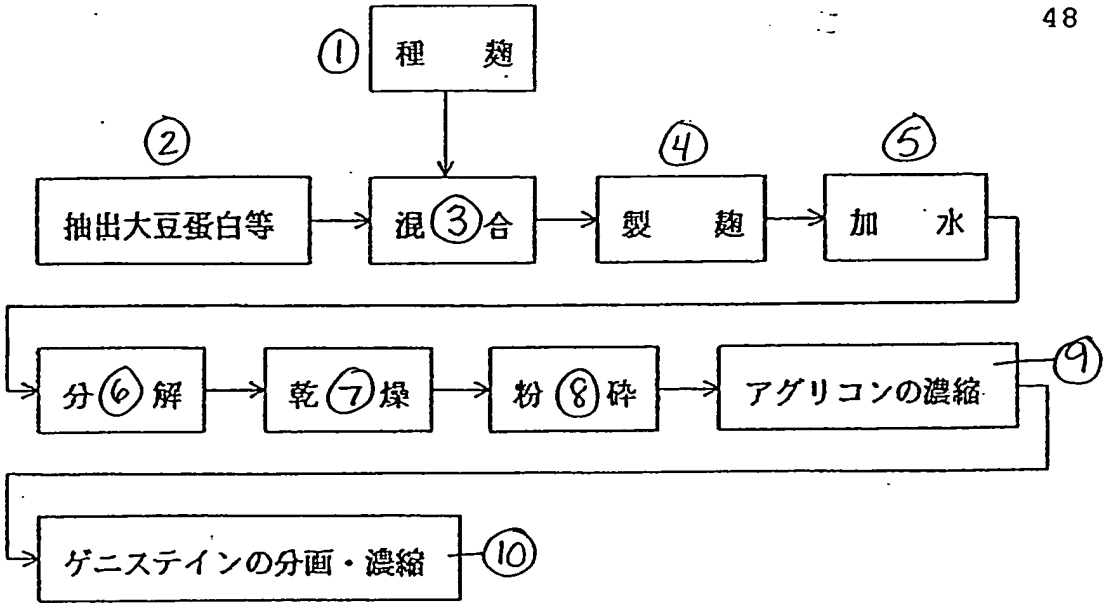
[0094]

Also, the present invention has the effect that allows for extremely easily and inexpensively preparing materials containing isoflavonoid aglycones, in particular, genistein, in high concentrations, and these materials can be used as the starting materials for pharmaceutical products using genistein as the main ingredient, and, moreover, it makes it possible to produce materials that allow to easily and inexpensively prepare pharmaceutical products containing genistein in high concentrations.

Brief description of the figures

Figure 1

A flow chart that shows Application Example 1 of a process for producing, in accordance with the present invention, aglycones possessing a powerful pharmacological action among the isoflavone compounds contained in soybean protein, and, simultaneously, shows Application Example 1 of a process for preparing products obtained by removing phytic acid from soybean protein.



Key: 1 Koji fungus seeding
2 Extracted soybean protein
3 Mixing
4 Koji preparation
5 Hydrolysis
6 Decomposition
7 Drying
8 Pulverizing
9 Aglycone concentration
10 Separation and concentration of genistein fraction